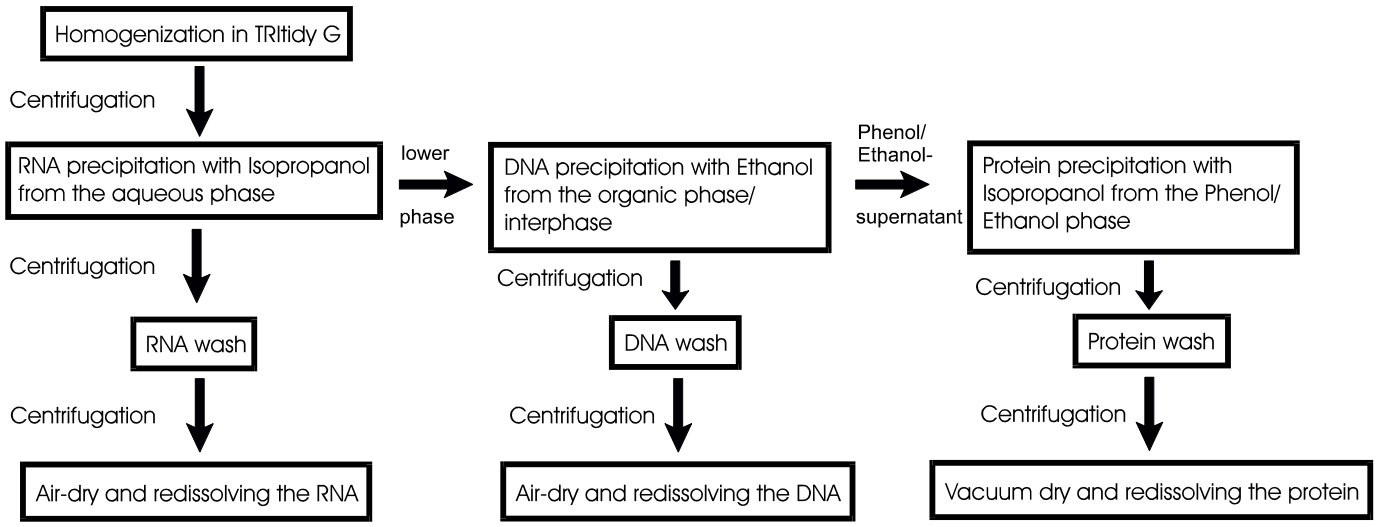
TRItidy G™

ready-to-use - solution for the simultaneous isolation of RNA, DNA and proteins
Product No. A4051

Description

The simultaneous isolation of RNA, DNA and proteins from biological samples has been introduced by Chomczynski (1) and improved by others. Chomczynski's method is based on the isolation of non-degraded nucleic acids (RNA and DNA) with guanidinium salts and phenol (e. g. ref. 2-5).

TRItidy G™ is a reagent, based on the Chomczynski method (1), with additional modifications to improve the purity of the RNA, DNA and proteins. First, the RNA will be selectively retained in the aqueous phase during the acidic GuaSCN/Phenol extraction, while DNA and proteins stay in the organic phase and interphase, respectively (6). The DNA is isolated from the interphase/ organic phase by a simple ethanol precipitation and proteins from the remaining organic phase.



Protocol

I.) RNA isolation:

I.1.) (a.) Tissue is homogenized in approx. 1 ml TRItidy G™ per 50 - 100 mg tissue (max. 1/10 of the volume of TRItidy G™). (b.) Cell culture cells are lysed in 1 ml/10^2 cm (3.5 cm diameter) dish, after aspiration of the medium. (c.) Cells, growing in suspension, have to be collected by centrifugation before addition of the reagent (1 ml TRItidy G™ per 1-5 x 10^6 cells; bacteria up to 1 x 10^7). (d.) For blood samples*, serum or other biological fluids add 750 µl of TRItidy G™ per 250 µl of sample volume.

*__Note:__ Biological fluids with high levels of protein or other contaminating substances (e.g. whole blood) may be diluted 1:1 with RNase-free, molecular biology grade water.

Homogenize the sample by pipetting the suspension up and down several times. A short incubation period improves the separation of RNA / protein complexes. Add 0,2 ml of chloroform to the lysate or, alternatively, 0,1 ml of 1-Bromo-3-chloropropane and mix well. Another incubation of 10 minutes additionally improves the purity of the RNA.

I.2.) Centrifuge the lysate at 12.000 g for 15 minutes at +4°C.

I.3.) Transfer the upper, aqueous phase to a new reaction tube and add the same volume of isopropanol to precipitate the RNA on ice for at least 15 minutes. Keep the organic phase/interphase with the DNA/protein at +4°C.

I.4.) Centrifuge at 12.000 g for 15 minutes at +4°C.

I.5.) Wash the RNA with ethanol (70-80 %) by vortexing and subsequently with absolute ethanol (100 %). Centrifuge at 7500 g at +4°C or room temperature.

I.6.) Air-dry the RNA and dissolve in 20 µl DEPC-treated water.

II.) DNA isolation:

II.1.) If necessary, remove the residual aqueous supernatant from the interphase and organic phase from step I.3 completely. This will improve the purity of the DNA dramatically. By addition of 0,3 ml ethanol per originally employed 1 ml TRItidy G™, the DNA will be selectively precipitated. For mixing with the ethanol, repeated inversion of the reaction tube is sufficient. Incubate the mixture for approx. 5 minutes at room temperature.

II.2.) Centrifuge at 2.000 g for 5 minutes at +4°C.

II.3.) Remove the supernatant (phenol/ethanol) and keep the supernatant at +4°C for the subsequent protein purification.

II.4.) Wash the DNA precipitate in 1 ml 0,1 M sodium citrate/10 % ethanol (per 1 ml TRItidy G™ reagent used in the original homogenization). Incubate for 30 minutes at room temperature; mix from time to time.

II.5.) Centrifuge at 2.000 g for 5 minutes at room temperature.

II.6.) Repeat step II.4. and II.5.

II.7.) Air-dry the DNA and dissolve in approx. 0,5 ml 1X TE (10 mM Tris, 1 mM EDTA; pH 8,0). Remaining cell fragments in this mixture are sedimented by centrifugation (12.000 g/10 minutes), while the DNA stays in solution under these conditions. Transfer the DNA-containing supernatant to a new reaction tube.

III.) Protein isolation:

III.1.) Proteins are precipitated from the phenol/ethanol mixture from step II.3. (DNA isolation). Add the double sample volume of isopropanol. Proteins will be precipitated during an incubation of approx. 10 minutes at room temperature.

III.2.) Centrifuge at 12.000 g for 10 minutes at +4°C.

III.3.) Wash the protein precipitate with 2 ml of 300 mM guanidine hydrochloride in 95 % ethanol per 1 ml TRItidy G™ reagent used for the initial homogenization. Incubate for 20 minutes at room temperature.

III.4.) Centrifuge at 7.500 g for 5 minutes at +4°C.

III.5.) Repeat twice step III.3. and III.4.

III.6.) Air-dry or vacuum-dry the protein precipitate, after removal of the supernatant. Dissolve the precipitate in 1 % SDS. Warming up to 50°C might be necessary. If you detect residual cell fragments or other insoluble components, remove them by an additional centrifugation step (10.000 g/10 minutes/+4°C). Transfer the supernatant to a new reaction tube (storage -20°C).

Caution: TRItidy G™ contains Phenol and Guanidinium thiocyanate. Please read the safety phrases on the label before use.

Application and Literature

(1) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. (Chomczynski, P. (1993) *BioTechniques* **15**, 532-537)
(2) The Use of Guanidinium Chloride in the Isolation of Nucleic Acids. (Cox, R.A. (1968) *Methods Enzymol.* **12 Part B**, [103a] 120-129)
(3) Isolation of Nucleic Acids with Phenolic Solvents. (Kirby, K.S. (1968) *Methods Enzymol.* **12 Part B**, [98] 87-99)
(4) Isolation of RNA with Guanidinium Salts. (MacDonald, R.J. *et al*. (1987) *Methods Enzymol*. **152**, 219-227)
(5) Co-existance of Vinculin and a Vinculin-like Protein of Higher Molecular Weight in Smooth Muscle. Modification of the GTC-Method for the RNA Isolation according to Chirgwin. (Feramisco, J.R. *et al*. (1982) *J. Biol. Chem.* **257**, 11024-11031)
(6) Large and Small Scale Phenol Extraction. (Wallace, D.M. (1987) *Methods Enzymol.* **152**, 33-41)
(7) Substitution of Chloroform by Bromo-chloropropane in the Single-step Method of RNA-Isolation. (Chomczynski, P. & Mackey, K. (1995) *Anal. Biochem.* **225**, 163-164)
(8) Modification of the TRI Reagent™ Procedure for Isoaltion of RNA from Polysaccharide- and Proteoglycan-Rich Sources. (Chomczynski, P. & Mackey, K. (1995) *BioTechniques* **19**, 942-945)
(9) RNA Extraction from Gastrointestinal Tract and Pancreas by a modified Chomczynski and Sacchi Method. (Monstein, H.-J. *et al*. (1995) *BioTechniques* **19**, 340-344)
(10) An Improvement of the Single-Step Method of RNA Isolation by acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. (Puissant, C. & Houdebine, L.-M. (1990) *BioTechniques* **8**, 148-149)
(11) Modified acid guanidinium thiocyanate-phenol-chloroform RNA extraction method with greatly reduced DNA contamination. (Siebert, P.D. & Chenchik, A. (1993) *Nucleic Acids Res.* **21**, 2019-2020)
(12) Effect of pH on RNA Degradation During Gunanidinium Extraction. (Noonberg, S.B. *et al*. (1997) *BioTechniques* **19**, 731-733)
(13) A Method for Sequential Extraction of RNA and DNA from the Same Sample, Specially Designed for a Limited Supply of Biological Material. (Chevillard, S. (1993) *BioTechniques* **15**, 22-24)
(14) Simultaneous Purification of RNA and DNA from Liver using Sodium Acetate Precipitation. (Evans, J.K. *et al*. (1998) *BioTechniques* **24**, 416-418)
(15) Isolation of HIV-1 RNA from Plasma: evaluation of eight different extraction methods. (Verhofstede, C. *et al*. (1996) *J. Virol. Methods* **60**, 155-159)
(16) Optimization of the Isolation and effective use of mRNA from rat mast cells. (Gilchrist, M. *et al*. (1997) *J. Immunol. Methods* **201**, 207-214)
(17) Comparison of Five Different RNA Isolation Methods from Equine Endometrium for Gene Transcription Analysis. (Kurar et al. (2010) Kafkas Univ Vet Fak Derg **16**, 851-855)